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Review Article

Relationships between PTEN and clinicopathological features of hepatocellular carcinoma: a meta-analysis

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Abstract: We performed a meta-analysis to demonstrate the relationship between PTEN expression in immunochemical staining and clinicopathological features of hepatocellular cancer (HCC). There were 11 eligible researches encompassing 872 HCC patients from the databases we used, including PubMed, Embase, and Web of Science. We are using the odds ratios (ORs) with 95% confidence intervals (95% CIs) to evaluate for absence of heterogeneity in a fixed effects model. Significant associations between positive PTEN expression and high tumor grades (OR = 2.56, 95% CI: 1.89-3.47, P < 0.00001), early tumor stage (OR = 3.26, 95% CI: 1.48-7.22, P < 0.00001), less cirrhosis (OR = 0.61, 95% CI: 0.42-0.89, P = 0.01) were found. However, according to the results, we found no significant associations between PTEN expression and gender, tumor size and capsule invasion of HCC patients.

Keywords: PTEN, clinicopathological features, hepatocellular carcinoma, meta-analysis

Introduction

Hepatocellular carcinoma (HCC), accounts for about 90% of all primary liver cancers worldwide, is one of the most common malignant tumor in China [1, 2]. To our knowledge, there are nearly one million new HCC patients diagnosed and 750 thousand deaths in 2012 [3]. Hepatocellular carcinoma (HCC) accounts for about 85 percent of primary liver cancers, which is concerned as the most commonly diagnosed histologic type [4]. It is wide accepted that genetic and epigenetic alterations are pivotal factors in the progression of HCC.

Phosphatase and tensin homologue on chromosome 10 (PTEN), one of the most frequently mutated tumor suppressors, only second to p53 [5], is a tumor suppressor gene located on human chromosome 10q23.3 [6, 7]. And it is worldwide accepted that PTEN may correlate with tumor proliferation, invasion, metastasis, and differentiation through the PI3K/Akt signaling pathway. Recently, the regulation of PTEN expression has been paid more attention. A series of PTEN mutations have been identified in several tumor types, including those of the gastric carcinomas, cervical neoplasm and the breast and cancer cell lines from various tissues [6-8]. According to the previous studies, we found that PTEN play the pivotal role in the development of HCC. However, the relationships between PTEN expression and clinicopathological features of HCC remain controversial. Whether PTEN is applied to classify subgroups of HCC patients more accurately? Then, we performed a meta-analysis to identify the relationship between PTEN expression and clinicopathological features of HCC.

Materials and methods

Search strategy

Electronic databases, include Web of science, the PubMed, and Embase were systematically searched online to find the relevant studies before May 1st, 2016. The keywords for the search included: “hepatocellular cancer” or “hepatocellular tumor” or “hepatocellular carcinoma” or “hepatocellular neoplasm” or “liver cancer” or “liver tumor” or “liver carcinoma” or “liver neoplasm” or “HCC” and “PTEN” or “phosphatase and tensin” or “MMAC1” or “TEP1”. Furthermore, we also manually searched the relevant references of selected studies to get

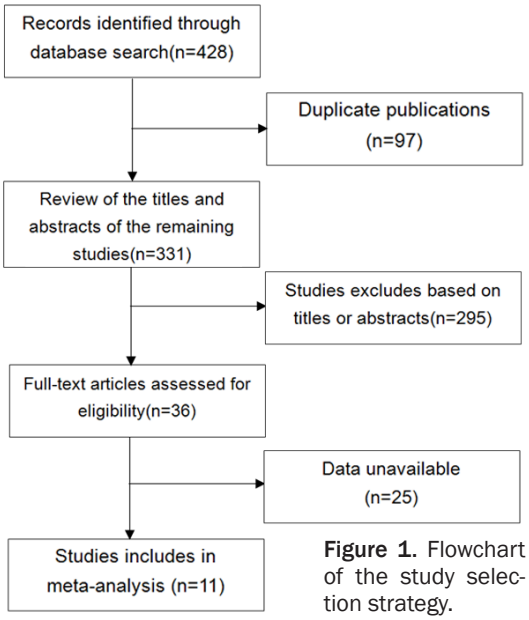

Figure 1. Flowchart of the study selection strategy.

some more potential researches. All authors' names and affiliations were attentively screened to avoid repeated data.

Selection criteria of the studies

The titles and abstracts of all publications were independently assessed by two reviewers (ZYD and HLQ). The further assessment was done by the third reviewer (SZC) through discussion with the first two authors. Studies were accepted if they met the following criteria: (1) Histopathologic information of HCC patients were confirmed by the pathologist review; (2) The expression of PTEN was performed in immunochemical staining, not in western blotting or PCR of HCC patients; (3) Data with regard to the relationships between PTEN and clinicopathological features of HCC patients was estimated by the pooled ORs and 95% CIs; (4) The data fit for estimating odds ratios (ORs) was chosen when duplications exist; (5) The tumor stages was assessed by TNM stage system; (6) Each individual study involved two treatment groups, namely, the HCC and control groups; (7) Positive PTEN expression was categorized into two indexes: 1. Proportion index: the proportion of PTEN protein positive cells were more than 10% of total cells; 2. Intensity index: the higher PTEN expression in HCC tissues than that in their adjacent normal tissues; (8) the population of this study was Chinese and Taiwanese.

Moreover, the following criteria were applied during the research: (a) non-original papers, such as conference abstracts, letters, and reviews; (b) duplicate publications; and (c) studies without qualified data or with 30 patients or less.

Data extraction

The eligible studies were independently extracted by two authors, the following data were extracted according to these criterion: first author, year of publication, country, number of patients, basic characteristics of the included patients, clinicopathological features, assay method of PTEN expression, number of patients with high PTEN expression and number of patients in case and control groups.

Quality assessment

The Newcastle-Ottawa Scale (NOS) criteria was applied to assess the quality of the eligible studies [9]. The NOS scores were calculated in following criterions: selection, comparability, and outcome, and a score of > 6 means high quality.

Statistical analysis

Review Manager software version 5.1 was used to perform the analysis and graphic of article. A fixed or random effects model was applied to estimate the combined ORs and 95% CIs. A heterogeneity test was used in analysis of heterogeneity of studies, including PTEN expression and Hepatocellular Carcinoma statistic and I^2 test, and a $p < 0.05$ or a I^2 result > 50% suggests significant heterogeneity. In this case, the random effects model was chosen; The fixed effects model was used in the lack of heterogeneity [10]. Funnel plots was applied to test Publication bias while Peters test was used to assess further statistically [11]. The trim and fill method was applied to adjust the combined ORs and 95% CIs when publication bias existed. To identify explore the influence of each single study on the overall estimate, sensitivity analysis was utilized to conduct the meta-analysis by STATA 12.0 (version 12.0, Stata Corporation) with the "meta" package.

Results

Study selection and quality assessment

The initial search identified 428 relevant studies, 97 of which were duplicates. The titles and abstracts of all studies were screened based

Table 1. Basic characteristics of the included studies

Study	Country	No. of case	Gender (M/F)	Age (Years)	Method (s)	Study design
Zhang TY 2004	China	47	41/6	52 (32-73)	IHC	Cohort study
Cheng Li-Ming 2003	China	62	-	-	IHC	Cohort study
Chen Jing-Song 2009	China	200	183/17	50.14±11.81	IHC	Cohort study
Sze Karen Man-Fong 2011	Hong Kong	40	31/9	34-74	IHC	Cohort study
Li Wang 2007	China	56	-	-	IHC	Cohort study
Su Rujuan 2016	China	110	85/25	50.02±15.67 (30-79)	IHC	Cohort study
Wu Shu-Kun 2007	China	31	27/4	43.45±10.77 (24-78)	IHC	Cohort study
Hu-1 Tsung-Hui 2007	Taiwan	124	100/24	55.8±11.9 (25-81)	IHC	Cohort study
Hu-2 Tsung-Hui 2003	Taiwan	105	84/21	55.7±12.2 (25-81)	IHC	Cohort study
Wan XW 2003	China	60	49/11	49.5	IHC	Cohort study
Li Xue-Feng 2003	China	37	-	-	IHC	Cohort study

IHC, immunochemical staining.

Table 2. Summary of pooled results in the meta-analysis

Groups	No. of studies	OR and 95% CI	P	Heterogeneity		Model
				I^2 (%)	P	
Gender	5	1.29 [0.83, 2.02]	0.26	67	0.02	Random
Tumor Grades	11	2.56 [1.89, 3.47]	0.00	28	0.18	Fixed
Tumor Stage	6	3.26 [1.48, 7.22]	0.00	0	0.53	Fixed
Tumor size	6	1.47 [0.96, 2.25]	0.29	48	0.09	Random
Cirrhosis	5	0.61 [0.42, 0.89]	0.01	0	0.68	Fixed
Capsule invasion	5	0.98 [0.69, 1.39]	0.89	63	0.03	Random

on inclusion/exclusion criteria, a total of 295 were excluded because of various reasons. After careful review by the author, we have 36 article and 25 studies were excluded for insufficient data. Then 11 articles were found to performed further analysis [5, 12-21]. The flow diagram of articles selection is summarized in **Figure 1**. There were 872 HCC patients involved in this meta-analysis, these 11 studies were published from 2003 to 2016 and were of good quality, with an average NOS score of 6.1. The basic characteristics of the 11 included studies are extracted and summarized in **Table 1**. A fixed-effects model was applied due to absence of heterogeneity, and the random effects model was performed for significant of heterogeneity. The summary of our meta-analysis results was showed in **Table 2**.

Our results showed that a high possibility was existed in the tumor grades (OR = 2.56, 95% CI = 1.89-3.47, p < 0.00001) (**Figure 2B**). We also evaluated the PTEN expression in the cirrhosis which include HCV or HBV related, and our finding revealed that there was significant association that high PTEN expression was related to a low possibility of cirrhosis (OR = 0.61, 95% CI = 0.42-0.89, p = 0.01) (**Figure 2E**). Additionally, according to the evidence we calculated, we confidently inferred that more positive PTEN expression found in I-II tumor stages than III-IV tumor stages (OR = 3.26, 95% CI = 1.48-7.22, p < 0.00001) (**Figure 2C**).

Then, we try to explore that whether positive PTEN expression was related with gender, and our results showed that no significant relationship between the gender and PTEN expression in HCC patients (p = 0.02, I^2 = 67%) (**Figure 2A**). The tumor size and capsule invasion were considered in this study, due to the two separate meta-analysis, we drew a conclusion that neither tumor size nor capsule invasion were obviously associated with PTEN expression (p = 0.09, I^2 = 48% and p = 0.02, I^2 = 70% respectively) (**Figure 2D**, **2F**).

The publication bias was assessed by funnel plots (**Figure 3**), and as the results showed that the plots of the publications were accepted, means no obviously significant biases. Meanwhile, we undertook a sensitivity analysis to identify the influence of each single study on the overall estimate (**Figure 4**). And we found that the sensitivity analysis was quite accepted.

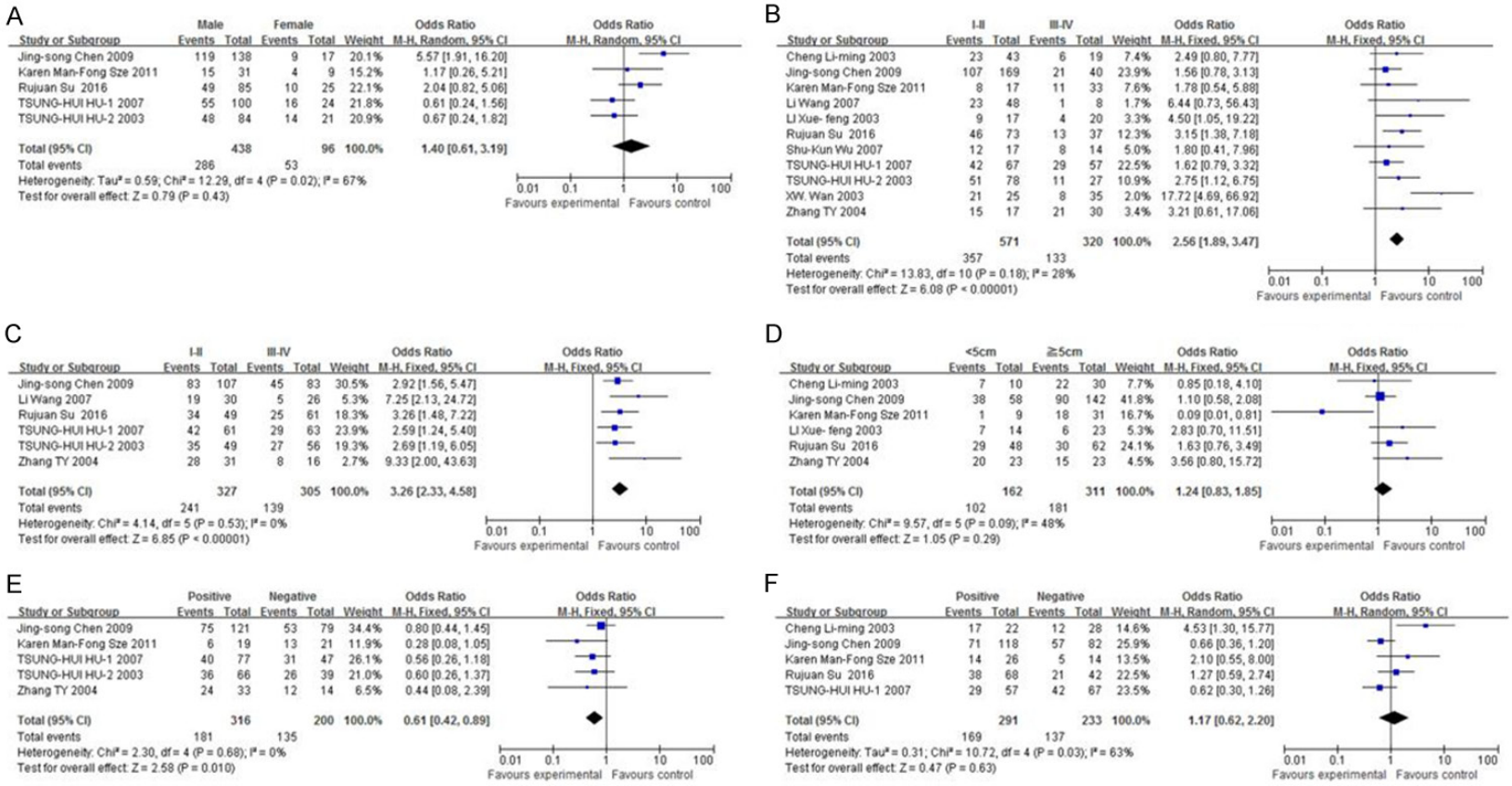

Figure 2. Forest plots for the relationships between PTEN and the clinicopathological features of HCC. A. Gender (male vs. female); B. Tumor grades (I & II vs. III & IV); C. TNM stage (I & II vs. III & IV); D. Tumor size (< 5 cm vs. ≥5 cm); E. Cirrhosis (positive vs. negative); F. Capsule invasion (positive vs. negative).

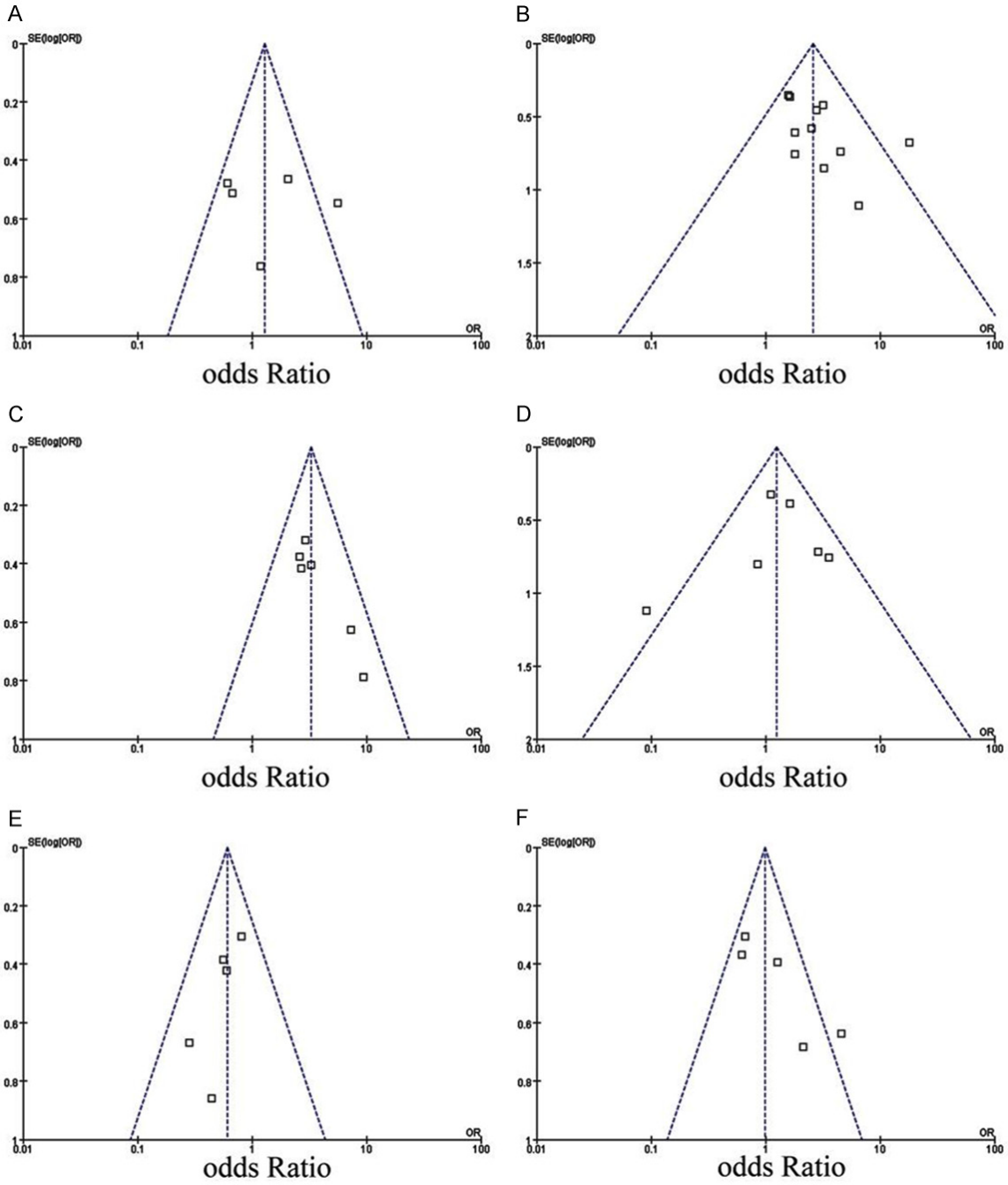

Figure 3. Funnel plots of publication bias. A. Gender (male vs. female); B. Tumor grades (I & II vs. III & IV); C. TNM stage (I & II vs. III & IV); D. Tumor size (< 5 cm vs. ≥5 cm); E. Cirrhosis (positive vs. negative); F. Capsule invasion (positive vs. negative).

Discussion

PTEN (phosphatase and tensin homologue deleted on chromosome ten) is one of the most mutated tumor suppressor implicated in a most part of human cancers [22]. PTEN encodes 403 amino acid polypeptides originally described as a dual-specificity protein phosphatase [23]. PTEN is constitutively expressed and a major negative upstream regulator of the PI3K/Akt signaling pathway [24, 25]. PTEN possesses a carboxy-terminal, noncatalytic regulatory domain with three phosphorylation sites (Ser380, Thr382, and Thr383) that regulate

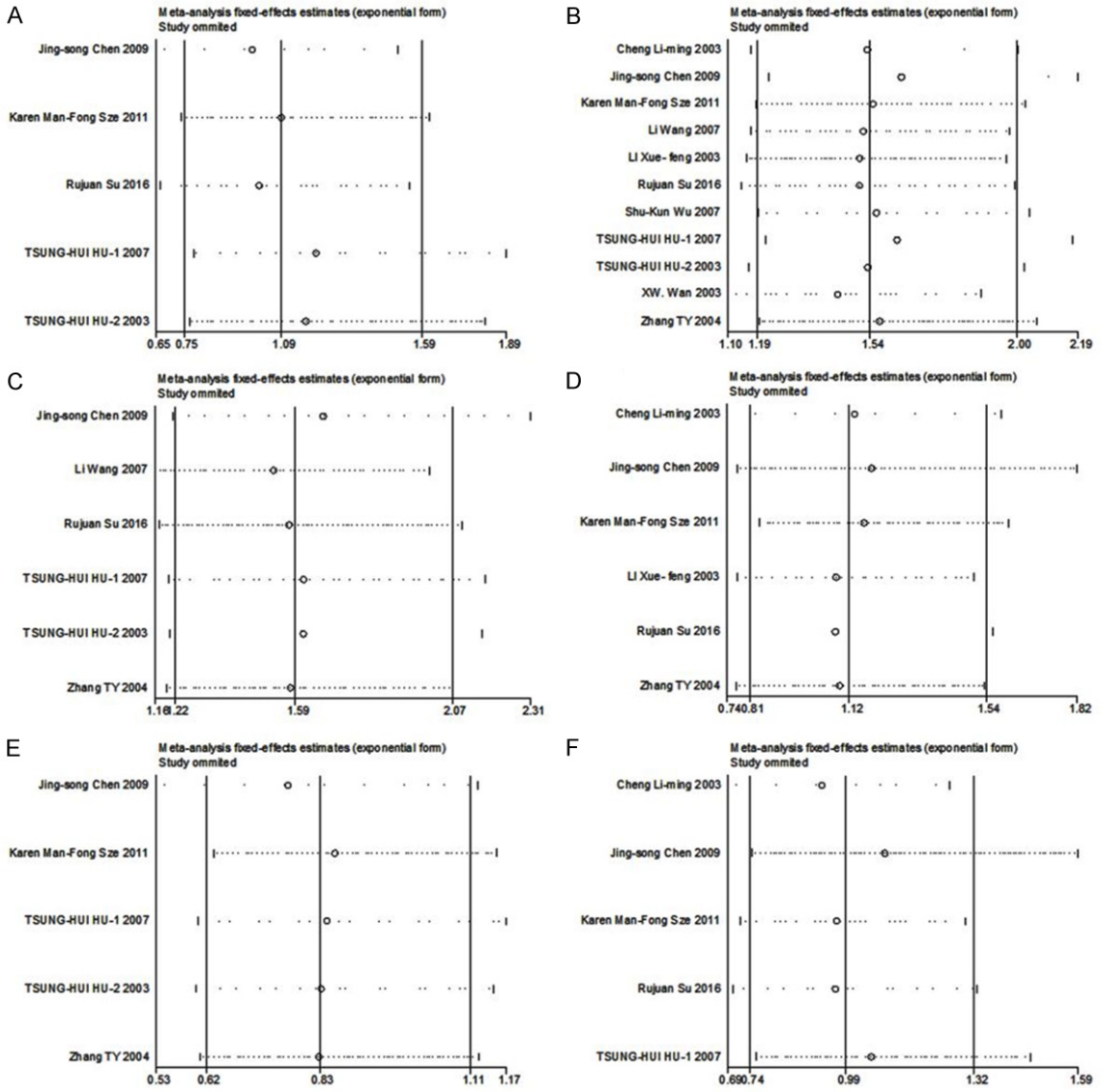

Figure 4. Sensitivity analysis of the influence of omitting each single study on the combined ORs. A. Gender (male vs. female); B. Tumor grades (I & II vs. III & IV); C. TNM stage (I & II vs. III & IV); D. Tumor size (< 5 cm vs. ≥5 cm); E. Cirrhosis (positive vs. negative); F. Capsule invasion (positive vs. negative).

PTEN stability and may affect its biological activity. PTEN regulates p53 protein levels and activity and is involved in G protein-coupled signaling during chemotaxis.

HCC accounts for about 110,000 deaths each year in China, with the 2nd most common cause of mortality among all malignant tumors. Mutations of PTEN gene in human HCC have been examined by many groups in worldwide. And immunohistochemistry was deemed as an accurate, mature and widespread method to identify the pathological features of HCC. The aim of this study was to reveal the relationships between PTEN and clinicopathological features of hepatocellular carcinoma.

In our study, we explored whether PTEN was associated with some clinicopathological features of HCC patients through 11 included studies. The gender, and capsule invasion were taken into the consideration, and the results revealed that there was significant heterogeneity existed ($p = 0.02$, $I^2 = 67\%$, $p = 0.02$, $I^2 = 70\%$ respectively), meanwhile, the funnel plot present that the publication bias were existed in these clinical features. It is hard to infer that PTEN is associated with gender, and capsule

invasion in HCC patients. The more studies may be explored to identify the all questions.

For investigating whether the tumor size was associated with PTEN expression, we performed the meta-analysis to clear this question. Our results revealed that the heterogeneity in this analysis was accepted ($p = 0.09$, $I^2 = 48\%$), but the statistical significance was unsatisfactory ($p = 0.29$). Although many previous studies showed that the loss of PTEN function may accelerate tumor proliferation, and was related with the bigger tumor size. This result suggested us that loss of PTEN expression may not play a role in the hepatocellular carcinoma proliferation. Whether the PTEN expression is not the key regulator in the process of tumor proliferation, more correlational research were needed.

We investigated the associations between PTEN expression and tumor grades. Our results demonstrated that the high expression level of PTEN was significantly associated with the absence of tumor thrombus and satellite lesion and histological grading in 60 cases of HCC ($P < 0.00001$). It was believed that a primary mode of PTEN inactivation may be at the level of transcription. There are several potential mechanisms that might explain the high PTEN mRNA expression [26] in the better tumor grades.

Simultaneously, we found that early tumor stages were associated with positive PTEN expression in HCC patients. According to the previous studies, Mutation of PTEN is a common event in advanced stages of diverse human malignancies, occurring in approximately 70% of patients with glioblastoma, 50% of patients with endometrial carcinoma, 50% of patients with prostate carcinoma, and 30% of patients with melanoma [27]. And the relationship between loss of PTEN and advanced tumor stages should be taken into consideration.

Additionally, low PTEN expression was more likely to be present in tumors with cirrhosis than those did not, in accordance with an immunohistochemically report on PTEN expression in tissues from HCV positive, cirrhotic patients with HCC [28]. It is well established that the correlation of PTEN and cirrhosis was taken seriously.

All funnel plots did not show any obvious asymmetry (**Figure 3**). Besides, sensitivity analysis was also performed to evaluate the power of every single study to influence the stability of conclusions. And, we are glad to find that the results of sensitivity analysis were quite stable.

To carry out this meta-analysis more scientifically, a comprehensive search method and well defined selection criteria were applied to obtain the eligible studies. Considering the influence of heterogeneity between studies, the Cochran's Q-statistic and I^2 test were used to choose a fixed or random effects model. To reduce the bias, publication bias was also estimated by funnel plots and sensitivity analysis was also performed to evaluate the influence of each single study on the overall estimate.

However, limitations should also be noted in this meta-analysis. Firstly, the possibility of information and selection biases could not be completely avoided because of the included studies were retrospective. Second, some included studies did not well predefine the inclusion criteria for patients, which might have influenced our results. Last, according to the characteristic of immunochemical staining, PTEN expression was categorized as negative (-) or positive (+) because it was difficult to quantify its staining intensity, which might have influenced our results.

In conclusion, our meta-analysis proved that positive PTEN expression may be obviously associated with high tumor grades, early tumor stages and no cirrhosis. And these results may support the previous studies and be applied in the clinical application to classify subgroups of HCC patients more accurately.

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Disclosure of conflict of interest

None.

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